



EFFECT OF *Hibiscus tiliacus* LEAF MEAL IN CONCENTRATE FEED ON NUTRIENT CONSUMPTION AND MICROBIAL PROTEIN SYNTHESIS IN THE RUMEN OF GOATS

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ABSTRACT

This study aims to determine the effect of *Hibiscus tiliaceus* leaf meal (HLM) supplementation in concentrate feed on nutrient consumption and microbial protein synthesis in the rumen of etawah crossbred goats. Sixty healthy male goats with an average initial body weight of 18.22 ± 3.09 kg were used in a completely randomized block design experiment, divided into four treatment groups. The four treatments were: (i) the goats were fed with elephant grass *ad libitum*+concentrate without HLM as much as 1% of body weight/head/day as control (H0); concentrate containing 5%; 10% and 15% of HLM as H1; H2, and H3, respectively. The results showed that HLM supplementation in the concentrate had a significant effect ($P < 0.05$) on protein and energy consumption. Likewise, microbial protein synthesis was significantly increased ($P < 0.05$) in the presence of HLM supplementation in concentrate feed. It can be concluded that supplementation of 5% *Hibiscus tiliaceus* leaf meal in the concentrate significantly increases energy and protein consumption, as well as microbial protein synthesis in the rumen which will affect the growth of goats.

KEYWORDS: Defaunation, protozoa, protein, purine, rumen, goat.

INTRODUCTION

Goats are one of the ruminants that are cultivated by farmers in rural areas. Maintenance of goats in general is still done traditionally and is part-time. Feeding relies more on the availability of fodder that grows around it. Forage forage generally has a high crude fiber content with a low digestibility value. To increase feed consumption and microbial protein synthesis in the rumen, it is necessary to provide concentrates containing defaunating agents for rumen microbial manipulation (France and Dijkstra, 2005).

In ruminants, the fermentative digestive capacity in the rumen reaches 70% of the entire digestive system. This is considered to have the greatest contribution to the overall feed digestibility process. Rumen conditions that are conducive to feed fermentation, degradation of fiber by microbes, nutrient consumption, and microbial protein synthesis for protein supply in ruminants will occur optimally (Calsamiglia et al., 2007).

Microorganisms found in the rumen of ruminants consist of protozoa, bacteria, fungi, and viruses. The existence of

these rumen microbes is very useful because they are able to utilize nitrogen instead of protein, digest large amounts of crude fibrous feed and produce rumen fermentation products that are easily absorbed in the intestines of ruminants (Wahyuni et al., 2014). Bacteria and fungi play a greater role in helping digestion of feed in the form of crude fiber, on the other hand, protozoa act as predators for bacteria. The digestion process in ruminants is mostly determined by fermentative digestion in the rumen. Ruminants are able to digest and utilize fibrous feed as a source of energy and nutrition, because they have the reticulum as an ecosystem where anaerobic microbes live, consisting of bacteria, fungi and protozoa (Durand and Osa, 2014; Punia et al., 2015).

Optimal rumen conditions will greatly affect fermentation for microbial protein synthesis in the rumen (Setiyaningsih et al., 2012). In order for the rumen ecosystem conditions to be more conducive to bacterial growth, it is necessary to control the protozoan population by providing feed containing saponins which function as defaunation agents. Defaunation is reported to be able to increase the growth and weight gain of

ruminants, especially if the feed given has a low nutrient content (Newbold et al., 2015).

Protozoa are a type of ciliate, namely the order *Prostomatida*, *Trichostomatida* and the largest number of *Entodiniomorphids*. The population in the rumen is 10^4 - 10^6 cells/ml of rumen fluid (Mackie et al., 2000), and its size is larger than the population of bacteria. Protozoa have the ability to digest cellulose and carbohydrates from grains (Wereszka and Michalowski, 2012). Protozoa in the rumen act as fiber digesters similar to fungi, but protozoa also prey on smaller size bacteria (McDonald et al., 2010). The predatory nature of protozoa against bacteria is a disadvantage in the fermentation system in the rumen. Protozoa prey on bacteria to meet the needs of amino acids in the synthesis of cell proteins. Protozoa tend to be stuck in the rumen and contribute less to microbial protein in the small intestine (Wahyuni et al., 2014).

Saponin content can reduce the protozoan population, so that at the same time it can increase the bacterial population in the rumen (Goel et al., 2008; Santoso et al., 2007). One of the forages that contain saponins is *Hibiscus* leaves. Utilization of *Hibiscus* leaves was able to reduce 32.31% of protozoa and increase 11.24% of bacteria in the ruminants' rumen compared to those without waru leaves (Putra, 2006). The administration of 200 ppm *Hibiscus* leaf extract with rice bran carrier media, was able to reduce 57.69% of the rumen protozoa population in vitro higher than that of cassava flour and ammoniated rice straw, namely 25.29% and 32% (Bata and Rahayu, 2016). Giving in vitro molasis solution containing *Hibiscus* leaf extract was reported to be able to increase feed consumption, dry matter digestibility and organic matter (Dinata and Pujiawati, 2018). Molasis

solution supplementation containing water extract of *Hibiscus* leaves as much as 10 cc/liter of drinking water in cattle fed elephant grass and polard as much as 1.5 kg/head/day can increase body weight gain 30.53% higher and improve fiber digestibility roughly compared to controls (Dinata et al., 2019).

Based on the description above, in an effort to increase nutrient consumption and microbial protein synthesis in the rumen, it is necessary to give *Hibiscus tiliaceus* leaf flour as a defaunation agent to suppress the protozoa population while increasing the bacterial population.

MATERIAL AND METHODS

Animal treatments and experimental design. Sixty healthy male goats with an average initial body weight of 18.22 ± 3.09 kg were used in a completely randomized block design experiment, divided into four treatment groups. The four treatments were: (i) the goats were fed with elephant grass *ad libitum*+concentrate without *HLM* as much as 1% of body weight/head/day as control (H0); concentrate containing 5%; 10% and 15% of *HLM* as H1; H2, and H3, respectively. The ingredients and chemical compositions of experimental diets were shown in Table 1.

Daily dry matter consumption (DM) was calculated by: the difference between the ration given and the remaining ration for each individual. To determine the nutrient content of the ration, a proximate analysis was carried out (AOAC, 2005) on the sample ration, then the amount of nutrients consumed can be known by the following calculations:

Nutrient consumption (g/day) = total feed intake (g/day) x %DM of feed x % of feed nutrients.

Table 1: The Ingredient and Calculated Nutrient Content of The Feed In Growing Goats.

Ingredients (%):	Concentrate*				Forage
	H0	H1	H2	H3	Elephant grass
Pollard	82	77	72	67	-
Rice bran	10	10	10	10	-
<i>Hibiscus</i> leaf meal	0	5	10	15	-
Molasses	5	5	5	5	-
CaCO ₃	1	1	1	1	-
NaCl	2	2	2	2	-
Mineral B12	0.5	0.5	0.5	0.5	-
Nutrien (% DW) ***					
Dry matter (%)	88.73	88.08	88.05	88.10	13.80
Organic matter (%)	85.35	84.63	84.44	84.23	75.42
Crude protein (%)	18.64	16.79	18.69	21.27	10.35
Ether extract (%)	9.73	9.02	9.49	10.20	5.75
Crude fibre (%)	3.81	6.08	6.26	3.32	24.80
Gross energy (kcal/g)	1.4749	2.4335	2.4784	2.2112	0.8301

* The goats were fed with elephant grass *ad libitum*+concentrate without *Hibiscus* leaf meal as much as 1% of body weight/head/day as control (H0); concentrate containing 5%; 10% and 15% of waru leaf meal as H1, H2, and H3, respectively.

** The mineral composition of B12 per 10 kg contains: Calcium: 49%; Phosphor 14%; Iron: 40000 mg; Manganese: 27500 mg; Mg: 27.500 mg; Zincum: 25 mg; Vit-B12: 4.50 mg and Vit D3: 500000 IU. PT. Eka Farma. Deptan RI No. D 8109127 FTS

*** Based on calculation according to Scott *et al.* (1982)

Analysis of the nutritional content of feed consisting of dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), extract ether (EE) and gross energy using the AOAC method (2005). Determination of the gross energy content (GE) of feed was carried out using an automatic adiabatic bomb calorimeter.

Rumen fluid samples were taken 4 hours after the goats were fed at the end of the total collection period, using a plastic pipe equipped with a mechanical suction pump. The method of taking rumen fluid was as follows: first prepare tools in the form of a plastic hose with a size of 3/8" with a length of 250 cm, a larger plastic hose with a size of 1/2 mm with a length of 40 cm, a mechanical suction pump, erlenmeyer divorced, 500 ml capacity bottles, sample bottles and filters. The vacuum pump was assembled with an erlenmeyer and from the erlenmeyer a 5/14" tube was installed which was inserted into the mouth of the goat. The end of the plastic tube was inserted into the mouth of the goat until it reaches the reticulo rumen with the protection of a larger tube to prevent the bite of the teeth. Furthermore, the suction pump was drawn repeatedly so that the rumen fluid was sucked out and immediately accommodated in the Erlenmeyer divorce. After sufficient rumen fluid was obtained, suction is stopped, and the plastic tube is pulled out.

Rumen microbial synthesis: Rumen microbial synthesis (RMS) can be calculated using the formula according to Chen and Gomes (1995) as follows:

$$\text{RMS (g/day)} = \text{Microbial nitrogen} \times 6.25$$

Microbial Nitrogen (MN) = 32 g/kg digestible organic matter in the rumen.

Digestible organic matter in the rumen (kg/day) = consumption of organic matter x digestibility of organic matter x 6.25

For a value of 6.25 = approximate number of fermentative digestibility in the rumen

Purine absorption (mmol/day) = MN: 0.727

Purine derivative excretion (mmol/day) = 0.85 purine abs + 0.385 x W^{0.75}

Alantoin excretion (mmol/day) = 0.85 x purine derivative excretion

Statistical analysis

The data obtained from the results of this study were analyzed by means of analysis of variance using the Costat Program with an error rate of 5% and if the variance test showed a significant difference (P<0.05), then the test was continued with Duncan's multiple range test.

RESULTS

The research results are presented in Table 2. HLM supplementation in concentrate feed had no significant effect (P>0.05) on consumption of elephant grass, concentrate, dry and organic matter, crude fiber, and ether extract. However, this showed a significant difference (P<0.05) in energy and protein consumption. Goats in Groups H1, H2, and H3 consumed significantly higher energy (P<0.05) compared to goats in Group H0 (control). The highest protein consumption was found in Group H3 goats, namely as much as: 77.88 g/head/day, which was significantly higher (P<0.05) compared to Group H1 and H2 goats, but not significantly different (P>0.05) compared to Group H0 goats.

Table 2: Effects of HLM supplementation in concentrate feed on nutrients consumption in PE goats.

Variable	Groups ¹⁾			
	H0	H1	H2	H3
<i>Consumption of nutrients</i>				
Elephant grass (g DM/head/day)	344.22 ^a ±58.87 ²⁾	330.01 ^a ±58.09	323.08 ^a ±28.22	359.62 ^a ±92.71
Concentrate (g DM/head/day)	184.50 ^a ±28.41	183.02 ^a ±28.07	171.42 ^a ±16.00	191.17 ^a ±44.79
Dry matter (g DM/head/day)	528.72 ^a ±86.70	513.03 ^a ±83.34	494.94 ^a ±42.75	550.79 ^a ±136.38
Organic matter (g DM/head/day)	417.07 ^a ±68.17	403.78 ^a ±65.27	388.41 ^a ±33.61	432.24 ^a ±106.74
HLM (g DM/head/day)	0	9.09 ^a ±1.39	17.10 ^a ±1.63	23.14 ^a ±5.65
Gross energy (kcal/head/day/h)	557.85 ^a ±90.09	719.31 ^b ±112.30	693.01 ^b ±60.96	721.21 ^b ±174.39
Crude protein (g DM/head/day)	70.01 ^{ab} ±11.30	64.87 ^a ±10.33	65.47 ^a ±5.70	77.88 ^b ±10.94 ²⁾
Crude fibre (g DM/head/day)	92.40 ^a ±15.65	92.97 ^a ±15.89	90.88 ^a ±7.88	95.53 ^a ±24.43
Extract ether (g DM/head/day)	37.74 ^a ±6.10	35.48 ^a ±5.66	34.22 ^a ±3.03	40.18 ^a ±9.81

Note:

- The goats were fed with elephant grass *ad libitum* + concentrate without HLM as much as 1% of body weight/head/day as control (H0); concentrate containing 5%; 10% and 15% of HLM as H1; H2, and H3, respectively.
- Mean of values with the same superscript on the same row, not significantly different (P>0.05)

The highest protein consumption occurred in Group H3, namely: 16.71% and 15.93% significantly ($P < 0.05$), higher than Group H1 and H2, and not significantly different ($P > 0.05$) rather than Group H0. Goats in Group H1, H2 and H3 consume energy, namely: 28.94%; 24.23%; and 29.28% significantly ($P < 0.05$) higher than the group H0 goats (Table 2.)

The effect of HLM supplementation in concentrate feed on rumen microbial synthesis (RMS) was presented in

Table 3. HLM supplementation in concentrate has no significant effect ($P > 0.05$) on digestible organic matter, N-microbes, purine absorption, excretion of purine derivatives, and allantoin excretion. However, microbial protein synthesis was significantly increased ($P < 0.05$) in the presence of HLM supplementation in the concentrate. Microbial protein synthesis in the rumen of Goats Group H1, H2, and H3, increased by: 19.98%; 19.47%; and 32.39% significantly ($P < 0.05$) higher than Group H0.

Table 3: Effect of HLM supplementation in concentrate feed on rumen microbial protein synthesis in PE goats.

Variables	Groups ¹⁾			
	H0	H1	H2	H3
Digestible organic matter (kg/day)	0.21 ^a ±0.06 ²⁾	0.19 ^a ±0.05	0.19 ^a ±0.02	0.21 ^a ±0.04
N-microbes (g/head/day)	6.60 ^a ±1.78	5.99 ^a ±1.59	5.96 ^a ±0.63	6.61 ^a ±1.31
Microbial protein synthesis (g/head/day)	31.18 ^a ±1.14	37.41 ^b ±1.96	37.25 ^b ±1.92	41.28 ^b ±2.19
Purine absorption (mmol/day)	4.79 ^a ±1.30	4.35 ^a ±1.16	4.33 ^a ±0.46	4.8 ^a ±0.95
Excretion of purine derivatives (mmol/day)	7.57 ^a ±1.64	7.15 ^a ±1.45	6.98 ^a ±0.58	7.67 ^a ±1.40
Allantoin excretion (mmol/day)	6.44 ^a ±1.39	6.08 ^a ±1.23	5.94 ^a ±0.49	6.52 ^a ±1.19

Note:

1. The goats were fed with elephant grass *ad libitum* + concentrate without HLM as much as 1% of body weight/head/day as control (H0); concentrate containing 5%; 10% and 15% of HLM as H1; H2, and H3, respectively.
2. Mean of values with the same superscript on the same row, not significantly different ($P > 0.05$)

DISCUSSIONS

Secondary metabolite compounds in plants have a taste that is less preferred by goats, so they can affect consumption and growth performance (Montoro et al., 2011). In general, dry matter (DM) consumption in all treatments was not significantly different (Table 2). This shows that the addition of HLM containing tannins and saponins does not have a negative effect on feed palatability. The limit of tannin concentration in feed is 50 g/kg DM (Patra and Saxena, 2009), while saponins are 40 g/kg DM (Patra and Saxena, 2011). This study is in line with Hu et al. (2005) who reported that the use of saponins derived from tea leaves had no effect on feed consumption, total VFA and partial VFA in the rumen.

According to France and Dijkstra (2005), the amount of feed consumption will affect the concentration of volatile fatty acids (VFA) in the rumen. Higher energy consumption will increase microbial protein synthesis in the rumen. The maximum growth of rumen bacteria as a result of the presence of saponins in HLM can lead to higher microbial protein synthesis to be used as a source of protein for host livestock. As reported by Liu et al. (2013), that HLM contains tannins which have orphyrobial properties through inhibitory action on microbial cell membranes, especially protozoan cells. Also reported by Kamra et al. (2006) stated that feeding with high tannin doses will reduce the population of methanogens, protozoa ciliates, and microbes that break down fibers in the rumen. Generally, tannins have antimicrobial activity that is more effective against gram-positive bacteria than gram-negative bacteria (Smith and Mackie, 2004).

By decreasing the population of protozoa in the rumen, it will reduce competition for the utilization of starch and dissolved sugars, so that the number of amylolytic bacteria will increase (Nagaraja, 2016). It was reported by Santoso and Hariadi (2007) that saponins are toxic not only to protozoa, but also to bacteria in the rumen. According to Patra and Saxena (2009), saponins also contain quinoline compounds, a type of antibiotic that can interfere with bacterial activity.

Microbial protein synthesis, associated with digestible organic matter in the rumen. The value of digestible organic matter is linear with the consumption of organic matter (Table 3) and the degradation of organic matter in the rumen of goats. According to Pineiro-Vazquez et al. (2015), giving HLM containing tannins, will reduce the digestibility of degraded proteins in the rumen and increase non-degraded proteins in the rumen. This results in reduced protein degradation in the rumen, even though the availability of protein in the feed is relatively higher.

CONCLUSION

It can be concluded that supplementation of 5% *Hibiscus tiliacus* leaf meal in the concentrate significantly increases energy and protein consumption, as well as microbial protein synthesis in the rumen which will affect the growth of goats.

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REFERENCES

1. AOAC. Official Methods of Analysis. 18. New York, AOAC International, 2005.
2. Bata, M dan Rahayu, S. Study of *Hibiscus tiliaceus* leaf extract carrier as additive in the diets for fattening of local cattle (in vitro). Pakistan Journal of Nutrition, 2016; 15(11): 969-974.
3. Calsamiglia, S. M., Busquet, P. W., Cardozo, L., Castillejos and Ferret, A. Invited Review: Essential oils as modifiers of rumen microbial fermentation. J. Dairy Sci., 2007; 90: 2580-2595.
4. Chen, X. B dan Gomes, M. J. 1995. Estimation of microbial protein Supply to sheep and cattle based on urinary excretion of purine derivatives. An overview of the Technical Details. UK: International feed resources Unit. Rowett Research Institute, Buckburn Aberdeen AB2 9SB.
5. Dinata, A. A. N. B. S and Pujiastuti, Y. *In Vitro* Study – Addition of Moladef in ration to rumen fermentation, dry matter and organicmatter digestibility. Proceedings International Conference of Bioscience and Biotechnology. Faculty of Agriculture Udayana University, Denpasar, 2018; 198-208.
6. Dinata, A. A. N. B. S., Pujiastuti, Y. and Aurum, F. S. Productivity of Bali cattle fed ration supplemented by molasses containing several types of defaunation Agents. JITV, 2019; 24(2): 49-54..
7. Durand, F. C and Ossa, F. The Rumen Microbiome: Composition, Abundance, Diversity, and New Investigative Tools. The Profesional Animal Scientist, 2014; 30: 1-12.
8. France, J. and Dijkstra, J. 2005. Volatile fatty acid productions. In: Quantitative aspect of Ruminant Digestion and Metabolism. 2nd Ed. CAB. International, Cambridge, USA.
9. Goel, G., H. P. S. Makkar and Becker, K. Changes in microbial community structure, methanogenesis and rumen fermentation in response to saponin-rich fractions from different plant materials. Journal Applycation Microbiology, 2008; 105: 770-777.
10. Goel, G and Makkar, H. P. S. Methane Mitigation from ruminants using tannins and saponins. Trop. Anim. Health Prod., 2012; 4: 729-739.
11. Hu, Wei-lian., Yue-ming, W., Jian-xin, L., Yanqiu, G and Jun-an, Y. Tea saponins affect in vitro fermentation and methanogenesis in faunated and defaunated rumen fluid. J. Zhejiang Univ. Sci., 2005; 6(8): 787-792.
12. Kamra, D. N., Agarwal, N and Chaudhary, L. C. Inhibition of ruminal methanogenesis by tropical plants containing secondary compounds. Int. Congress Series, 2006; 1293: 156-163.
13. Liu, X. L., Hao, Y. Q., Jin, L., Xu, Z. J., Mcallister, T. A and Wang, Y. Anti-*Escherichia coli* O157:H7 properties of purple prairie clover and sainfoin condensed tannins. Molecules, 2013; 18: 2183-2199.
14. Mackie, R., Aminov, R., White, B and McSweeney, C. Molecular Ecology and Diversity in Gut Microbial Ecosystems. In: Ruminant Physiology. Digestion, Metabolism, Growth and Reproduction. CAB Int., Oxford, UK., 2000; 61-77.
15. McDonald, P., Edward, R. A., Greenhalgh, J. F. D., Morgan, A., Sinclair, L. A and Wilkinson, R. G. Animal Nutrition. Seventh Edition..Harlow-England: Pearson, 2010.
16. Montoro, C., Ipharraguerre, I., Bach, A. Effect of favoring a starter in a same manner as a milk replacer on intake and performance of calves. Anim. Feed Sci. Technol, 2011; 164: 130-134.
17. Nagaraja, T. G. Microbiology of the rumen. in rumenology. Cham: Springer International Publishing, 2016; 39-61.
18. Newbold, C. J., de la Fuente, G., Belanche, A., Ramos-Morales, E and McEwan, N. The role of ciliate protozoa in the rumen. Front. Microbiol, 2015; 6: 1-14.
19. Patra, A. K., Kamra, D. N and Agarwal, N. Effect of plant extracts on *In Vitro* methanogenesis, enzyme activities and fermentation of feed in rumen liquor of buffalo. Anim. Feed Sci. Technol, 2006; 128: 276-291.
20. Patra, A. K and Saxena, J. A Review of the effect and mode of action of saponins on microbial population and fermentation in the rumen and ruminant production. Nutr. Res. Rev., 2009; 22: 204-219.
21. Patra, A.K and Saxena, J. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. J. Sci. Food Agric, 2011; 91: 24-37.
22. Pineiro-Vázquez A. T., Canul-Solis J. R., Alayon-Gamboa, J. A., Chay-Canul, A. J., Ayala-Burgos, A. J., Aguilar-Perez, C. F., Solorio-Sanchez, F. J and Kuvera, J. C. Potential of condensed tannins for the reduction of emissions of enteric methane and their effect on ruminant productivity. Arch. Med. Vet., 2015; 47: 263-272.
23. Punia, A. K., Singh, R., and Kamra, D. N. Rumen Microbiology: from Evolution to Revolution. India: Springer, 2015.
24. Santoso, B. and Hariadi, B. T. Effect of supplementation of *Acacia mangium* Willd in *Pennisetum purpureum* on fermentation characteristic and methane production in vitro. Med. Pet, 2007; 30(2): 106-113.
25. Santoso, B., Kilmaskossu, A and Sambodo, P. Effects of saponin from *Biophytum petersianum* klotzsch on ruminal fermentation, microbial protein synthesis and nitrogen utilization in goats. Animal Feed Science Technology, 2007; 137: 58-68.
26. Setyaningsih, K. D., Christiyanto, M dan Sutarno. Kecernaan bahan kering dan bahan organik secara *in vitro* hijauan *Desmodium cinereum* pada berbagai dosis pupuk organik cair dan jarak tanam. J. Animal Agriculture, 2012; 1(2): 51-63.

27. Smith, A. H and Mackie, R. I. Effect of condensed tannins on bacterial diversity and metabolic activity in the rat gastro intestinal tract. *Appl. Envir. Microbiol*, 2004; 70: 1104-1115.
28. Wahyuni, I. M. D., Muktiani, A dan Christianto, M. Penentuan dosis Tanin dan Saponin untuk defaunasi dan peningkatan fermentabilitas pakan. *JITP*, 2014; 3(3): 133-140.
29. Wereszka, K., and Michalowski, T. The Ability of the rumen ciliate protozoan *diploplastron* affine to digest and ferment starch. *Folia Microbiologica*, 2012; 57(4): 375-377.